Supporting Information

One-step Coordination of Metal–Phenolic Networks as Antibacterial Coating with Sustainable and Controllable Copper Release for Urinary Catheter Applications

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Materials and methods

Surface characterization

To investigate the optimal reaction condition for complexation of TA and Cu ions, the reaction solution was prepared at various TA/Cu molar ratios (the total TA-Cu molar concentration was fixed at 3.76 mmol/L). A 1 cm × 1 cm × 0.1 cm PDMS substrate was immersed in 4 mL of the TA-CuSO$_4$ solution, and shaking in a constant temperature oscillator (THZ-98A, Bluepard) (100 rpm, 37°C). Full scan UV-Visible spectrum and absorbance at 291 nm of the coated PDMS were recorded using a UV–vis spectrophotometer (Cary-300, Agilent Technologies, USA). Surface morphology of uncoated PDMS, and TA, TC-1 and TC-2 samples was investigated using atomic force microscopy (AFM). The sample was stuck on a silicon wafer, and observed using an AFM (Bruker Fast Analyst, Bruker Co., Ltd.) at 0.994 Hz scan rate and 8 kHz peak force frequency. Water contact angle of the substrate surface was measured by Drop Shape Analyzer (DSA25B, KRÜSS Scientific). Four μL of deionized water was dropped on samples by a double-dosing unit at 20°C. Three replicates were measured for each group. For X-ray photoelectron spectroscopy (XPS) measurement, an AXIS SUPRA spectrometer (Kratos Analytical Ltd., Manchester, UK) with Al Kα X-ray source (1468.6 eV), and the binding energies were calibrated to the C 1s hydrocarbon peak at 284.8 eV. Fourier transform infrared (FTIR) spectra of pristine and TA- and TC-coated PDMS were measured using a Nicolet iS50 Spectrometer (Thermo Fisher Scientific, USA).

Characterization of TA-Cu complex

The complex of TA-Cu formed from TA-CuSO$_4$ solution at a TA/Cu molar ratio of 1:3 after reaction at 37°C for 24 h was collected by lyophilization. The sample was subjected to characterization using $^1$H nuclear magnetic resonance (AVANCE NEO, 400 MHz, Bruker, Germany, using dimethyl sulfoxide-d6 as the solvent) and liquid chromatography-quadrupole-time-of-flight mass spectroscopy (TripleTOF® 4600, AB Sciex, USA, using distilled water as the solvent).
Results and discussion

Fourier transform infrared spectra of TC coating

Figure S1 shows the FTIR spectra of pristine, and TA- and TC-coated PDMS substrates. Both of the TA and TC coatings showed adsorption bands at 1539 cm\(^{-1}\) and 1701 cm\(^{-1}\), caused by stretching vibration of C-O, and C=O bonds. The TA coating exhibited a broad absorption band at 3000-3700 cm\(^{-1}\) due to the high content of C\(_{\text{Ar}}\)-OH. The C\(_{\text{Ar}}\)-OH absorption band decreased in the TC coating, indicating that Cu ions chelated with the phenol groups of TA molecules.

\[\text{Figure S1. FTIR spectra of pristine PDMS, and TA- and TC-coated PDMS substrates.}\]

\(^1\text{H} NMR\) spectra of TA and TC complex

TA and TC complex were dissolved in DMSO-d6 and analyzed using \(^1\text{H} NMR\). The chemical shifts of 8.8-10.4 ppm, 6.7-7.7 ppm, and 4.2-6.6 ppm are attributed to the phenolic hydroxyls, aromatic protons, and protons in the glucoside ring, respectively (Figure S2). The relative area ratio of phenolic hydroxyls to glucoside protons of TC compound (1.47) decreased compared to that of TA (1.69), probably due to the chelation of phenolic hydroxyl groups of TA to copper ions.
Figure S2. $^1$H NMR spectra of TA and TC complexes in DMSO-d6.

**LC-Q-TOF MS measurement**

The mass spectra of TA and TC complexes were recorded at the m/z range of 200-2000 (Figure S3). The phenolic hydroxyl groups were easily ionized in electrospray ionization\(^2\). Ten peaks were identified as the potassium adduct ions of TA: 

- [glucose+\(C_7H_6O_4\)-H+K]\(^+\),
- [glucose+2\(C_7H_6O_4\)-2H+K]\(^+\),
- [glucose+3\(C_7H_6O_4\)-3H+K]\(^+\),
- [glucose+4\(C_7H_6O_4\)-4H+K]\(^+\),
- [glucose+5\(C_7H_6O_4\)-5H+K]\(^+\),
- [glucose+6\(C_7H_6O_4\)-6H+K]\(^+\),
- [glucose+7\(C_7H_6O_4\)-7H+K]\(^+\),
- [glucose+8\(C_7H_6O_4\)-8H+K]\(^+\),
- [glucose+9\(C_7H_6O_4\)-9H+K]\(^+\)

and

- [glucose+10\(C_7H_6O_4\)-10H+K]\(^+\) occurred at m/z 371.0381, 523.0494, 675.0627, 827.0735, 979.0853, 1131.0991, 1283.1112, 1435.1276, 1587.1241 and 1739.1798, respectively. Seven peaks were recognized as the potassium adduct ions of TC complex:

- [glucose+2\(Cu\)-2H+K]\(^+\),
- [glucose+\(C_7H_6O_4\)+4\(Cu\)-7H+K]\(^+\),
- [glucose+2\(C_7H_6O_4\)+4\(Cu\)-8H+K]\(^+\),
- [glucose+3\(C_7H_6O_4\)+4\(Cu\)-9H+K]\(^+\),
- [glucose+4\(C_7H_6O_4\)+4\(Cu\)-10H+K]\(^+\),
- [glucose+5\(C_7H_6O_4\)+4\(Cu\)-11H+K]\(^+\)

and

- [glucose+6\(C_7H_6O_4\)+4\(Cu\)-12H+K]\(^+\) occurred at m/z 344.2010, 619.0936, 771.1084, 923.1184, 1075.1327, 1227.1390 and 1379.1511. The change of a series of m/z peaks in the spectrum demonstrated Cu ions had strong affinity to chelate with the phenolic
hydroxyl groups of TA, and the formed TA-metal complexes were stable in water.

Figure S3. (a) Chemical structure of TA, and (b) positive ion ESI mass spectra of TA and TC complexes.

Optimization of reaction condition for TA-Cu complex

Reaction solution containing various molar ratio of TA and CuSO₄ (total molar concentration of TA and CuSO₄ fixed at 3.76 mmol/L) was used in TC coating preparation to explore the optimum coating condition. The TC coating showed a characteristic peak at 291 nm (Figure S4a). The Job’s plot (TA molar fraction versus absorbance of coating at 291 nm) exhibited an increase of the absorbance as the TA molar fraction increased from 0.125 to 0.25 (corresponding to TA/Cu molar ratio of 1:7 to 1:3), and further increase of TA molar fraction did not result in increase of the absorbance of the coating (Figure S4b). Therefore, the feeding ratio of TA/CuSO₄ at 1:3 was selected in the rest of the study for TC coating preparation.
Figure S4. (a) UV-Visible spectra of TA- and TC-coated PDMS. (b) Job’s plot of TC coatings prepared using different TA molar fraction in the TA-Cu mixture. The absorbance of TC-coated PDMS substrate was recorded at 291 nm (n=3). * denotes statistically significant difference with \( p<0.05 \), ** denotes \( p<0.001 \), and ns denotes no significant difference between the designated groups.

**Time-dependent antibacterial property of TC-2 coating**

The time-dependent antibacterial property of TC-2 coating against Gram-positive *S. aureus* and Gram-negative *E. coli* and *P. mirabilis* was investigated (Figure S5). Pristine and TC-2-coated PDMS substrates (1 cm × 1 cm × 0.1 cm) were incubated in 2 mL of PBS bacterial suspension (10^6 cells/mL) at 37°C for 24 h. At the time points of 0, 3, 6, 12, and 24 h, the viable bacteria in the suspension were counted by spread plate method. After 3 h of incubation, the killing efficiency of TC-2 coating against *S. aureus*, *E. coli* and *P. mirabilis* achieved 99.04%, 99.95%, and 97.39%, respectively. The TC-2 coating kills bacterial cells in the suspension in a time-dependent manner, and after 24 h of incubation, *E. coli* cells in the suspension were eliminated, and 99.99% of *S. aureus* cells and 99.81% of *P. mirabilis* cells were killed. The TC-2 coating with such efficient bacteria killing capability showed potential in antibacterial catheter application.
**Figure S5.** Time-dependent antibacterial property of TC-2-coated PDMS against *P. mirabilis*, *E. coli* and *S. aureus* after incubation for 0, 3, 6, 12, 24 h.

**Long-term biofilm formation**

Long-term bacterial colonization on the coatings over 72 h was investigated. The biofilm formed on the pristine and modified PDMS substrates was observed using SEM (Figure S6) and CLSM (Figure S7). As can be seen, after 72 h of culture, thick biofilm formed on the pristine PDMS surface and surfaces with TA coating. A high number of bacterial cells on the TC-1 coating was observed. In contrast, TC-2 coating remained relevantly clean with few bacterial cells, and thus showed strong suppression against biofilm formation.
Figure S6. SEM images of bacterial biofilm of *P. mirabilis, E. coli* and *S. aureus* on the pristine and modified PDMS surfaces after incubation for 72 h. Scale bars represent 30 μm.

Figure S7. CLSM images of bacterial biofilm of *P. mirabilis, E. coli* and *S. aureus* on the pristine and TC-2-coated PDMS surfaces after incubation for 72 h. Scale bars represent 100 μm.
Monocyte number and percentage

Blood from the animals after 7 days of catheter implantation was collected and subjected to the complete blood count test. The number and percentage of monocytes was detected as monocytes are indicative signals of inflammation due to acute infections (Figure S8). According to the World Health Organization (WHO), symptoms with absolute monocyte count ≥10⁹/L, monocytes percentage ≥10% of leukocytes and persisting for ≥3 months are diagnosed as persistent monocytosis. As can be seen, blood sample from animals of the control group showed high monocytes percentage (19.4% and 33.6%), while two blood samples of the TC-2-coated catheter group had high monocyte percentage (10.6% and 14.6%). Moreover, the blood samples from the control group had higher monocyte count than that from the TC-2-coated catheter group. The TC-2 coating reduced the risk of monocytosis during the acute infections and suppressed the inflammation.

Figure S8. Monocyte number and monocyte percentage were tested by the complete blood count (CBC) of rabbits with unmodified catheters and TC-2-coated catheters after 7 days of catheter implantation (n=5).

References
